Autologous Chondrocyte Transplantation for Disc Repair

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Introduction: The study was designed to assess whether autologous disc chondrocyte transplantation will retard disc degeneration following discectomy and additionally to determine whether transplanted cells are capable of stimulating matrix regeneration subsequent to injury. Cultured autologous cells were transplanted into the nucleus pulposus of eighteen dogs by a closed procedure using a small caliber spinal needle. While it has been shown that disc cells will sustain a phenotype in culture, the fate of re-implanted cells is less well understood.

Methods Used: Mature dogs were radiographed prior to surgery. Blunt dissection was used to reach the margin of the outer anulus, and 100 mg of nucleus and inner anulus material was sampled from two levels (L1-L2 and L3-L4). The L1-L2 disc of each dog served as a control, while the L3-L4 level was used as the site for transplantation. Although the L2-L3 site was approached, the anulus was not violated. Disc chondrocytes were propagated in culture and re-implanted 10 weeks later under fluoroscopic guidance. Disc samples obtained from healthy dogs were enzymatically digested and isolated chondrocytes were proliferated in monolayer culture. Cells were cultured for 5 weeks under GMP conditions that included 10% autologous serum. During the final 4 days of culture (passage 2), BrdU was added to the culture as a thymidine analogue for nuclear incorporation.

Subsequently, the culture was divided and one part of the cells was kept in ML, whereas the other part was transferred into a 3-D culture system. Cells of different passages in ML (P1, P2, P3) were grown on slides and cryosections of the aggregates after 3 weeks, 11 weeks, and 12 weeks were made. BrdU within the sections was stained by immunohistochemistry with a specific antibody and visualized by peroxidase reaction (Figure 1).

Figure 1. BrdU staining of cells in monolayer at varying passages to assure the retention of stain.

Growth curves of labeled and unlabeled cells of the same donor were performed to analyze the influence of the BrdU-labeling on the proliferative capacity and vitality of the cells. Post-operative radiographs were taken monthly, and the animals were euthanized at 3 months, 6 months, 9 months, and at 1 year. Intervertebral discs were assessed by histology, radiography, and MRI. Vertebral segments T-13 through S-1 were removed en bloc, radiographed, and prepared for MRI. Images were collected at 1.5T in both T1 and T2 weightings. Disc heights were calculated using a spine index that averaged the height of the disc relative to the two adjacent vertebrae.

Summary of Findings: MR images exhibited changes related to the surgical defect and reflected a loss of characteristic disc morphology. Although both experimental levels demonstrated less proton density than the unoperated control, the level receiving cell transplantation (L3-L4) demonstrated a positive difference in quantity and intensity of signal compared to the level that had not received cell transplantation. MRI changes were positive over time; showing enhanced central disc signal, and reduction in endplate effusion. The chief pathology evident from the MRI exam was a loss of intensity in the central portion of the L1-L2 intervertebral disc. Differences in disc height were measured according to a technique developed by Lu, et al. ANOVA of 14 dogs by time across levels was non-significant (F = 0.95; p = 0.42), but ANOVA by level across time was significant (F = 3.4; p = 0.04) indicating that the change in mean height was time dependent and that the 12-month dogs drove the change.

Multiple ANOVA (MANOVA) supports this finding as well. When disc height was analyzed by level, holding time as a constant co-variates, the difference is significant (F = 3.62; p = 0.036); however, this significance disappears if the 12-month dogs are excluded (F = 1.29; p = 0.289) as it does if the MANOVA looks at time, holding leve constant but without considering the 12-month interval (F = 0.226; p = 0.799). The trend of a longitudinal, evolving distinction between treatment levels, is affirmed with the addition of the 12-month data despite between-dog differences that remained as large as the difference between the levels.

Histology offered a clear impression of pathology. Vertebral bone at levels that had received cells were healthier in appearance as judged by red marrow, subchondral vertebral bone architecture, and the quality of the intervertebral disc itself. Cells that had been transplanted into the disc populate the disc space, made matrix, and retarded inflammation to a greater extent than did levels that had not received the cells (Figure 2).

Figure 2. Twelve months post-transplantation–Note repair of subchondral plate, normal disc height, and matrix regeneration.

It would be inaccurate to designate the new morphology as mirroring normal canine intervertebral disc, but the results of high matrix to cell ratio, the abundance of chondrocytes in the absence of cloning, and lack of vascularized inflammatory process were all positive interpretations of the success of this effort. The introduction of cells seemed to have a positive affect on cell height, MRI signal, and matrix appearance.

Relationship Between Findings and Existing Knowledge: Autologous cells transplanted into a damaged intervertebral disc appear to slow degeneration. Evidence of matrix production, suppressed inflammation, and cell viability was evident grossly (dissection), by MRI, radiography, by histology, and by nuclear marker. From these early results, autologous disc chondrocyte cell transplantation appears to offer the promise of retarding degeneration, demonstrates a trend in maintaining intervertebral height, and offers positive results that matrix regeneration after discectomy is a viable option.

Overall Significance of Findings: While cell viability, matrix production, and matrix integration suggest autologous cell transplantation may be a valuable clinical tool, refining technology will strengthen the value in application.

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